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The Effect of Site of Application of ^{14}C-Fluazifop on Its Uptake and Translocation by Quackgrass (*Agropyron repens*)[1]

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Abstract. **The effect of site of application on uptake and translocation of the butyl ester of fluazifop {(±)-2-[4-[[5-(trifluoromethyl)-2-pyridinyl] oxy] phenoxy] propanoic acid} by quackgrass [*Agropyron repens* (L.) Beauv. #[3] AGRRE] was investigated using ^{14}C-labeled herbicide and intact plants. Uptake and distribution of the label were significantly greater from the abaxial than from the adaxial surface of leaves. The addition of a nonionic surfactant[4] to the treatment solution increased the uptake significantly only through the adaxial surface. Uptake of ^{14}C by the apical, middle, and basal regions of the treated leaf lamina did not differ significantly. However, movement of the ^{14}C-label to stem areas and leaves both above and below treated leaves was greater from lamina base applications than from treatments to the lamina apex and middle. The older leaves absorbed more herbicide than did younger leaves, but the pattern of translocation did not differ. Considerably greater translocation occurred from treatments to the outside of the leaf sheaths in the lower regions of the stem than from applications to the upper leaf sheaths, with the ^{14}C-label moved to young and old leaves, roots, and rhizomes. Uptake from applications to the outside of the upper leaf sheaths also resulted in improved translocation mainly within the stem areas and into upper leaves.**

Additional index words. **AGRRE.**

INTRODUCTION

Quackgrass is a persistent perennial weed that is known to be extremely difficult to control because of its extensive underground rhizome system (16). Control of quackgrass depends upon killing this subterranean system, particularly the rhizome buds. Thus, herbicides that translocate well in the phloem are useful to control quackgrass. Fluazifop, a postemergence, selective herbicide used in the control of perennial and annual grass weeds in broadleaf crops (17) has performed efficiently and adequately in the control of quackgrass under a variety of conditions (4, 10, 13, 14). Fluazifop is translocated well to areas of high metabolic activity in susceptible plants including quackgrass, and the main pattern of translocation appears to be through the phloem, closely following the source-sink relationships of the plant (4, 5, 11, 13).

Various factors related to the plant, environment, and spray formulation affect the performance of postemergence herbicides (3, 8, 9, 15) by influencing uptake and translocation. The site of deposition of a postemergence herbicide can influence its phytotoxicity (6, 18). The growth stage of the plant or the relative age of the leaves that receive much of the foliar application has been known to influence uptake and translocation (1, 2). The greater permeability of the lower leaf surface (3, 12) is usually attributed to differences in the fine structure of wax on the adaxial surface (7) and to the presence of more stomata and trichomes. Mature fully expanded leaves generally absorb less of exogenously applied chemicals than immature leaves (3). Mature leaves are the principal sources of assimilates with which foliage-applied herbicides move in the translocation stream from source to sink. Young leaves do not export assimilates appreciably, but once they are a third to a half expanded, they export rather than import (19). Mature leaves near the base of a plant export assimilates to the roots while those near the top export to the apex. Leaves in the middle may export to both roots and tops (3).

The objective of this research was to investigate the effect of site of application of fluazifop on its uptake and translocation by quackgrass plants. Experiments were carried out to: a) compare abaxial and adaxial leaf surface treatments in the presence or absence of additional surfactant, b) determine the effect of placement of herbicide droplets at different locations of the same leaf, and c) compare uptake and translocation from treatments to different individual leaves and leaf sheaths.

MATERIALS AND METHODS

Growth of plants. Quackgrass plants were raised from rhizome material obtained from a clone maintained at the Penyffridd Field Station of the University College of North Wales in Bangor, Gwynedd, U.K. The rhizomes were cut into single-node pieces 25 to 30 mm long and planted in flat plastic trays (42 by 30 by 4 cm) containing washed quarry sand. They were covered with 1 cm of potting soil (1:1:1, soil:sand:peat) and placed in a heated glasshouse where the temperature varied between 18 and 22 C, and the relative humidity was 60 to 65%. Natural daylight was supplemented with 400-watt mercury vapor lamps providing a 16-h illumination of 250 $\mu E \cdot m^{-2} \cdot s^{-1}$ PPFD. When new plants had emerged and grown for about 14 days, they were transplanted singly into 9.5-cm-diam plastic pots filled with potting soil. Plants were watered as required daily.

Quackgrass plants for the experiments were 12 weeks old after transplanting into pots individually, and had seven

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[3] Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds, Weed Sci. 32, Suppl. 2. Available from WSSA, 309 West Clark Street, Champaign, IL 61820.

[4] 'AGRAL'—nonyl phenol ethoxylate, from I.C.I. Plant Protection, U.K.

to nine leaves on the main shoots and three to four well-developed tillers and rhizomes. Average height of plants was approximately 110 cm from the base of the plant to the tip of the youngest leaf. Before radioactive herbicide applications, plants were selected for uniformity.

^{14}C-treatment solution. ^{14}C-Fluazifop, labeled at the ester carbonyl atom and having a specific activity of 21.5 mCi/mmole, was used. An emulsifiable concentrate equivalent to commercial fluazifop, 250 g/l (680.5 mmole/l) but containing radiolabeled fluazifop, was prepared by adding appropriate volumes of a blank formulation[5], labeled herbicide and 100% technical grade fluazifop. Depending on the number of treatments, replicate plants to be treated, and treatment volumes per plant, the required volume of emulsifiable concentrate was prepared and diluted in deionized water to an emulsion containing 25 nCi/μl. The final herbicide concentration in the treatment solution was adjusted to 5.0 μg of fluazifop per μl (13.61 mmole/l).

Experimental treatments. Immediately before the application of radiolabeled herbicide, plants were sprayed with 0.5 kg ai/ha of unlabeled fluazifop in combination with 0.1% (v/v) surfactant, using an Oxford Precision Sprayer[6] fitted with an Allman No. 0 nozzle[7], at a pressure of 2.25 kg/cm^2. During this spraying the leaf or stem part to be treated later with radioactivity was kept covered using lightweight polyethylene film. After the spraying these covers were removed. The leaves to be treated with radioactivity were placed horizontally on a movable stage and held with small strips of sticky cloth tape.

The following three experimental treatments were made:

1. Uniform plants were treated with 0.125 μCi of ^{14}C-fluazifop from a treatment solution that had 0.025 μCi/μl of ^{14}C-fluazifop with or without the surfactant at 0.1% (v/v). Applications were made to the adaxial or abaxial leaf surface of the second youngest leaf between 5 and 15 cm from the ligule.

2. In the second experiment, a different set of plants was treated with ^{14}C-fluazifop at different positions on the same leaf. Treatments were made to the lamina apex (15 to 21 cm from ligule), lamina middle (8 to 15 cm from ligule), and lamina base (0 to 8 cm from ligule) on the adaxial surface of the second youngest leaf. Activity per plant was 0.125 μCi. The treatment solutions had the surfactant incorporated at 0.1% (v/v).

3. In the third experiment, the effect of treating different leaves and parts of the stem on uptake and translocation was studied. Applications of 0.125 μCi of ^{14}C-fluazifop were made to the following parts of quackgrass plants using different plants for each treatment: first, second, third, fourth, or fifth youngest leaves (L 1, L 2, L 3, L 4, or L 5, respectively), upper stem (between L 1 and L 2), or lower stem (between L 5 and oldest leaf below it). In the case of leaf applications, droplets were placed on the adaxial surface between 5 and 15 cm from the ligule. Stem applications were made to the areas between the specified leaves and were actually treatments to the outer surface of leaf sheaths. These treatments had the surfactant incorporated at 0.1% (v/v).

In all three experiments, the herbicide was delivered using a 1.0-μl microsyringe. Five μl of treatment solutions (0.025 μCi/μl) were applied per plant as ten 0.5-μl droplets. Applications were always in a row close to the midvein of the leaves. Each experiment had four replicate plants per treatment which were randomized after treatment. The temperature in the glasshouse was ca. 25 C, relative humidity 60 to 65%, and light intensity 250 $\mu E \cdot m^{-2} \cdot s^{-1}$. All plants were harvested for radioassay 3 days after herbicide applications.

Plant harvesting and ^{14}C-assay. In all three experiments the treated plants were separated into their component parts for radioassay. If the leaves had been treated, plants were separated into the treated leaf, leaves and stem above treated leaf, leaves and stem below treated leaf, roots, and rhizomes, and tillers. The treated leaf was divided into the treated zone, the areas above the treated zone, and the areas below it. In experiment 3, in which some of the treatments were to the stem, plants were separated into leaves and stem above the treated zone, leaves and stem below the treated zone, the treated zone itself, roots, rhizomes, and tillers. The unabsorbed ^{14}C-residue on the treated areas was washed with three 5-ml aliquots of deionized water, followed by dipping in 10 ml of n-hexane, and both the water and hexane rinses separately collected. These were radioassayed by adding 1-ml aliquots into 10 ml of a dioxane-based scintillant[8]. The leaves and other plant parts were freeze dried for 24 h, stored in paper bags, and later oxidized in a biological sample oxidizer[9]. The liberated $^{14}CO_2$ was collected in 15 ml of a scintillant cocktail containing a CO_2 absorbent[10] and radioassayed using liquid scintillation spectrometry. Where necessary, large plant parts were ground after drying and 50 or 100 mg subsamples used for radioassay.

Statistical treatment. All results were subjected to analyses of variance. The significance of treatment means was determined and, where effects were significant, comparison among means was done using Duncan's multiple range test.

RESULTS AND DISCUSSION

The recoveries of applied ^{14}C-activity in the three experiments were low and ranged from 32.7 to 37.2%, 45.3 to 65%, and 36.9 to 69.2%, respectively, in the three cases.

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[6] MDM Engineering (Portsmouth) Ltd., 34 Aston Rd.,Waterlooville, Hampshire, England, U.K.

[7] E. Allman & Co., Ltd., Birdham Rd., Chichester, Sussex, England, U.K.

[8] Dioxane-based Liquid Scintillator Fluid (NE 250) supplied by Nuclear Enterprises Ltd., Sighthill, Edinburgh EH11 4EY, Scotland, U.K.

[9] Harvey Biological Material Oxidizer Model OX 100, R. J. Harvey Instruments Corp., 123 Patterson Street, Hillsdale, New Jersey 07642, U.S.A.

[10] Scintillant cocktail containing NE 233 (supplied by Nuclear Enterprises), Phenylethylamine (CO_2 absorbent) and Ethanol at 1.7:1:1 (v/v), respectively.

In an earlier study (5) in which recovery of ^{14}C-activity was low from treated quackgrass plants, positive evidence of volatility losses from treated glass surfaces was obtained. Relatively rapid loss of ^{14}C-activity occurred in the first 6 h (42% of applied activity), and by the end of the experiment (48 h after treatment of cover slips) 62% of ^{14}C-activity initially applied had been lost, indicating that loss of ^{14}C-fluazifop due to volatilization from treated surfaces would be considerable (5). In the earlier study (5) and in the present studies, quackgrass plants relatively advanced in age and size, and having extensive rhizome systems, were used, since fluazifop would be required to control very well established infestations as well. However, another possible reason for the poor recovery of ^{14}C-activity could have been the use of these large plants, which necessitated much subsampling and possibly led to some underestimation of activity. Kells et al. (13), in their studies with ^{14}C-fluazifop and much smaller quackgrass plants than we used, also found that the recovery of ^{14}C-activity decreased with time after application. However, Hendley et al. (11) reported that recoveries of ^{14}C-fluazifop from treated plants did not decrease significantly until 14 days after treatment, although there were significant volatility losses from treated glass surfaces by 10 days after treatment. Other reasons that might have caused low recoveries would be the inability to recover all root hairs from the soil, the possibility of some exudation of radiolabeled herbicide or metabolites from roots into soil, $^{14}CO_2$ evolution after metabolism of the herbicide, and activity not recovered through leaf wash procedures (5).

The total recovery of ^{14}C-activity averaged over all treatments of the first experiment was 34.7% of the activity initially applied, with little difference between the treatments (Table 1). Without the surfactant, the uptake was significantly greater through the abaxial surface. The surfactant increased the uptake of the herbicide significantly only through the adaxial surface. Translocation of the ^{14}C-activity was significantly greater following treatments to the abaxial than to the adaxial surfaces. The radioactivity moved both acropetally and basipetally within the treated leaves and shoots. Since uptake from the adaxial surface increased in the presence of the surfactant, the differences in amounts translocated to various parts of the plants were reduced. The present results are in agreement with those of Veerasekaran, Kirkwood, and Fletcher (18) who reported enhanced uptake of ^{14}C-asulam {methyl[(4-aminophenyl) sulphonyl] carbamate} through abaxial leaf surfaces of bracken (*Pteridium aquilinum* L. Kuhn) in the presence of the surfactant Tergitol-7 (0.1%), compared with uptake through adaxial leaf surfaces.

In the second experiment, in which the recovery of applied ^{14}C-activity was ca. 50%, there were no significant differences among treatments with respect to recovery of the ^{14}C-label. The total uptake of herbicide by the plants also did not differ significantly among treatments (Table 2) and ranged from 16.6 to 18.9% of the total applied.

The activity that remained in the treated area was significantly greater following applications to the lamina apex, and correspondingly, the amount of ^{14}C-movement was least from this treatment. From treatments in the lamina middle and base, much of the activity moved out of the treated zone and was distributed both within and out of the treated leaf. Of the 8.7% of the radiolabel, which had remained within the untreated zone in lamina middle treatments, 6.1% had moved acropetally, and 2.6% basipetally (data not presented). With lamina base treatments, however, only 2.9% of the ^{14}C-label had moved acropetally in the same leaf (Table 2). Major differences in the total activity translocated out of the treated leaf, and in translocation patterns, were found with the lamina base applications, resulting in significantly large quantities of the ^{14}C-label in the leaves and stems above and below the treated leaf. The amount of the ^{14}C-label in roots and rhizomes was nearly the same with both lamina middle and lamina base applications and was considerably higher compared to lamina apex treatments. These results are in good agreement with the studies of Coupland, Taylor, and Caseley (6) who reported greater uptake and translocation of glyphosate [*N*-(phosphonomethyl)glycine] on quackgrass, when applications were made towards the leaf base. The authors suggested that variation in the amount of epicuticular wax among different areas of the same leaf could be an important factor determining how much chemical is absorbed, as might the microclimate in the leaf base area. The same explanation may relate to our results with fluazifop.

In the third experiment, the total recovery of applied ^{14}C-activity was in the range of 36.9 to 69.2% and again

Table 1. The effect of treating adaxial or abaxial surfaces of quackgrass leaves with or without surfactant, on uptake, translocation, and recovery of ^{14}C-fluazifop, 72 h after treatment.

Wash or tissue fraction	^{14}C recovered[a]			
	Adaxial surface		Abaxial surface	
	Herbicide alone	+ Agral (0.1%, v/v)	Herbicide alone	+ Agral (0.1%,v/v)
	(% of applied)			
Leaf wash	29.0 a	19.1 b	23.2 ab	24.0 ab
Activity in treated leaf:				
Treated zone	4.2 a	4.9 a	1.9 c	2.6 b
Above treated zone	1.0 b	6.2 a	3.5 a	4.8 a
Below treated zone	0.4 c	1.2 b	2.1 ab	3.6 a
Activity in rest of plant:				
Leaves and stem above	0.2 b	0.5 a	0.6 a	0.3 b
Leaves and stem below	0.3 b	0.4 b	1.7 a	1.4 a
Roots and rhizomes	0.3 c	0.4 b	0.3 c	0.5 a
Total recovery	35.4 a	32.7 a	33.3 a	37.2 a
Total uptake	6.4 b	13.6 a	10.1 a	13.2 a
Total translocation[b]	0.8 b	1.3 b	2.6 a	2.2 a

[a]Mean values within a row followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test.

[b]Total translocation refers to the activity recovered from the parts of the treated plant other than the treated leaf.

Table 2. The effect of treating different positions of the adaxial surface of the second youngest leaf (L 2), on the uptake, translocation, and recovery of ^{14}C-fluazifop, 72 h after treatment.

Wash or tissue fraction	^{14}C recovered		
	Lamina apex[b]	Lamina middle[b]	Lamina base[b]
	(% of applied)		
Leaf wash	46.1 a	26.9 b	33. 0 b
Activity in treated leaf:			
Treated zone	16.9 a	7.3 b	4.5 b
Untreated area	1.0 c	8.7 a	2.9 b
Activity in rest of plant:			
Leaves and stem above	0.2 b	0.5 b	2.2 a
Leaves and stem below	0.6 b	0.9 b	6.0 a
Roots and rhizomes	0.2 b	1.0 a	1.0 a
Total recovery	65.0 a	45.3 a	49.6 a
Total uptake	18.9 a	18.4 a	16.6 a
Total translocation[c]	1.0 b	2.4 b	9.2 a

[a]Mean values within a row followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test.

[b]Lamina apex: 15 to 21 cm, Lamina middle: 8 to 15 cm, and Lamina base: 0.8 cm distance from the ligule.

[c]Total translocation refers to the activity recovered from the parts of the treated plant other than the treated leaf.

varied significantly among treatments (Table 3). Uptake of ^{14}C-activity was greatest from leaves three and four (L 3 and L 4), compared to uptake from leaves one and two (L 1 and L 2). Uptake by leaf five, the oldest of the treated leaves, and from other treated regions of the stem, were intermediate. Activity translocated out of the treated leaves did not differ significantly between the treatments to different leaves and ranged from 1.3 to 2.1% of applied ^{14}C-activity. When expressed as percent of absorbed ^{14}C-herbicide, these values range from 11% (from L 5) to 13.5% (from both L 1 and L 4) and were still not statistically significant. Compared to leaf treatments, greater ^{14}C-translocation was evident from application to stem areas through leaf sheaths. Approximately 56 and 68% of absorbed activity was translocated from applications to upper and lower stem regions, respectively (Table 3). The greatest movement out of the treated zone was from applications to lower stem (mature stem tissue). Accumulation of the ^{14}C-label was primarily in upper leaves (2.0%), lower leaves (1.3%), and roots and rhizomes (1.3%). Following applications to the younger tissues of the upper stem, the ^{14}C-label was exported mainly to the upper leaves, but large quantities also remained in the stem tissues both above and below the treated area.

Movement of the ^{14}C-label out of the treated leaves was appreciably less with applications to the youngest leaves

Table 3. The distribution of ^{14}C-activity in quackgrass plants, 72 h after application of ^{14}C-fluazifop to different individual leaves, and upper and lower leaf sheaths.

Wash or tissue fraction	^{14}C recovered						
	Treated tissue						
	L 1	L 2	L 3	L 4	L 5	Upper sheath[b]	Lower sheath[b]
	(% of applied)						
Leaf/leaf sheath wash	55.3 a	38.0 cd	29.5 ef	42.9 bc	24.2 f	53.8 a	30.1 de
Activity in treated leaf or leaf sheath area:							
Treated zone	4.4 ab	2.7 c	3.1 bc	4.2 b	4.6 ab	6.8 a	3.9 bc
Above treated zone	3.3 b	6.0 ab	11.5 a	8.8 a	6.2 ab	. . .	. . .
Below treated zone	1.2 a	0.5 a	1.2 a	0.6 a	0.5 a	. . .	. . .
Activity in rest of plant[c]:							
Upper leaves	0.2 b	0.4 b	0.4 b	0.4 b	0.4 b	2.7 a	2.0 a
Lower leaves	0.2 b	0.1 b	0.4 b	0.1 b	0.1 b	0.1 b	1.3 a
Upper stem	0.5 b	0.3 bc	0.2 cd	0.2 cd	0.1 d	3.3 a	0.4 bc
Lower stem	0.2 d	0.3 cd	0.5 b	0.5 b	0.4 bc	2.1 a	3.3 a
Roots and rhizomes	0.3 de	0.2 e	0.5 c	0.9 b	0.4 cd	0.4 cd	1.3 a
Total recovery	65.6 a	48.2 bc	47.3 bc	58.6 ab	36.9 c	69.2 a	42.3 c
Total uptake	10.3 c	10.5 c	17.8 a	15.7 ab	12.7 bc	15.4 ab	12.2 bc
Total translocation[d]	1.4 b	1.3 b	2.0 b	2.1 b	1.4 b	8.6 a	8.3 a

[a]Mean values in a row followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test.

[b]Upper leaf sheath treatment to the outside of sheath area between L 1 and L 2; lower leaf sheath outside of sheath area between L 5 and leaf below.

[c]Upper leaves or stem mean leaves and stem above treated leaf or stem area. Lower leaves or stem mean leaves and stem below treated leaf or stem area.

[d]Total translocation refers to the activity recovered from the parts of the treated plant other than the treated leaf or stem region.

(L 1 or L 2), compared with applications to L 3 or L 4, although the differences were not statistically significant. In both young and mature leaves, significant quantities of the label that had penetrated were found to have moved acropetally towards the leaf tips. Applications to individual mature leaves (L 3 or L 4) caused significantly large quantities of the ^{14}C-label to be transported to lower stem regions, roots, and rhizomes, compared with treatments to L 1 or L 2 (Table 3). No clear trends could be seen with respect to movement of activity into upper and lower leaves from the individual leaf applications.

The results indicate that greater movement of ^{14}C-fluazifop occurred from applications directly to stem regions (leaf sheaths) than to individual leaves. This observation is in good agreement with the studies of Coupland et al. (6) and Wills (20) who reported enhanced uptake and movement from 'stem' applications than from leaf treatments. Wills (20) has suggested that more mature tissues absorb and translocate more herbicide than immature tissues. Evidence supporting this view was obtained with fluazifop treatments to the lower stem from which the ^{14}C-label was distributed more widely through the entire plant. Greater accumulation of the label in roots, rhizomes, and lower leaves may have been due to the close proximity of these areas to the treated lower stem.

Young leaves with less wax on their surface are usually regarded as more permeable to foliar-applied herbicides than older, more mature leaves (6). However, in the present studies, uptake from applications to the youngest leaves (L 1 and L 2) was found to be significantly lower than from L 3 and L 4. Since foliar absorption is believed to be a diffusion process, continued uptake would depend on the maintenance of a concentration gradient across the cuticle. Younger leaves are generally regarded as 'importers' of assimilates rather than 'exporters'. This would mean that foliar-absorbed herbicides, which depend to a greater or lesser extent on assimilate movement, may not be exported from such leaves in very great quantities, and this may be the explanation for lower uptake from the younger leaves in intact plants in the present studies.

It is clear that fluazifop moves in the phloem with the assimilate stream along 'source-sink' relationships of the plant (4, 11, 13). However, viewed in the light of the findings of Dewey and Appleby (8), who have recorded evidence of greater apoplastic movement of ^{14}C-glyphosate than previously thought, in addition to its well-confirmed phloem translocation, it is interesting to note the similarity of the behavior of fluazifop. Dewey and Appleby (8) recorded greater translocation of ^{14}C-glyphosate from stem applications than from leaf applications, a result in agreement with our studies. Also, glyphosate had moved readily in large amounts to all leaves above the treated tissue regardless of the stage of leaf maturity from stem applications. They argue that this ability to enter and accumulate in mature leaves above the point of application is characteristic of xylem-translocated herbicides, and suggest that much of the acropetal movement that occurred would have taken place in the transpiration stream. In our studies also large amounts of the ^{14}C-label were moved into leaves above the point of application from treatments to the lower stem via lower leaf sheaths. Dewey and Appleby (8) point out that if ^{14}C-glyphosate is applied to leaves, the apoplastic movement is towards the leaf tips and margins, but if applied to the stem, significant apoplastic distribution occurs into all transpiring tissues above that point. They argue the case for considerable apoplast-to-phloem transfer of glyphosate occurring within the treated leaf and throughout the plant and express the view that apoplastic movement also appears to play a significant role in determining overall distribution of foliar-applied herbicides within treated plants, especially if uptake takes place through stem areas. The results we have obtained with ^{14}C-fluazifop support this view.

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